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# An essential role for sodium in the bicarbonate transporting system of the cyanobacterium *Anabaena variabilis*

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The apparent photosynthetic affinity of *Anabaena variabilis* for extracellular inorganic carbon ($C_i$) was strikingly increased by $Na^+$. The effect was highly specific for $Na^+$ and was maximal at 40 mM $Na^+$. $Na^+$ supply decreased the apparent $K_m$ ($C_i$) of the $C_i$ transporting system and to a lesser extent increased $V_{max}$. It did not affect photosynthetic rate expressed as a function of intracellular $C_i$. We infer an effect of $Na^+$ on the $C_i$ transporting system rather than on the photosynthetic machinery itself. We propose several possible models, including $Na^+$–$H^+$ antiport for maintenance of intracellular pH during $HCO_3$ uptake, and $Na^+$–$HCO_3^-$ symport.

Anabaena *Inorganic carbon uptake* *Photosynthesis* *Sodium-proton antiport* *Sodium-bicarbonate symport*

## 1. INTRODUCTION

Active transport and accumulation of inorganic carbon ($C_i$) in cyanobacteria involves a primary electrogenic pump [1], but the molecular mechanism of transport has not yet been elucidated. Bicarbonate appears to be the $C_i$ species arriving at the inner side of the plasmalemma [2]. Since $CO_2$ is the species utilized by the carboxylating enzyme [3], hydroxyl ions must be released to the medium in order to maintain intracellular pH. Alkalization of the medium following the supply of $HCO_3^-$ has in fact been observed in *Anabaena* [4]. The mechanism involved in $OH^-$ efflux is not understood, but the efflux (or $H^+$ influx) appears to occur along its electrochemical potential gradient [5]. It has recently been suggested that an $Na^+$–$H^+$ antiport system is involved in the maintenance of intracellular pH in bacteria [6–8]. We have examined the possibility that a similar system may operate in cyanobacteria. Here, we demonstrate that $Na^+$ plays a mojor role in the mechanism for $HCO_3^-$ uptake in these organisms.

## 2. MATERIALS AND METHODS

Cells of *Anabaena vaiabilis* were grown as in [1,9], at a $CO_2$ level equal to that in air. Cells were harvested by centrifugation (500 × *g*, 5 min) and resuspended in 40 mM 1,3-bis(tris-hydroxymethylmethylamino)propane (BIP) brought to pH 9.0 with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes).

Photosynthetic $O_2$ evolution was measured in an $O_2$ electrode as in [1,9]. Accumulation of acid-stable and -labile $^{14}C$ and intracellular $C_i$ concentration were determined by a filtering centrifugation technique after the supply of $NaH^{14}CO_3$ [1,9].

## 3. RESULTS AND DISCUSSION

Fig.1. gives the photosynthetic rate as a function of external $C_i$ concentration in the presence and absence of $Na^+$ in the medium. It shows that the apparent photosynthetic affinity for $C_i$ was strongly affected by $Na^+$. Maximum photosynthetic rate at saturating $C_i$ level was also affected but to a considerably lesser extent (fig.1). It has been observed that apparent photosynthetic affinity for $C_i$ in the



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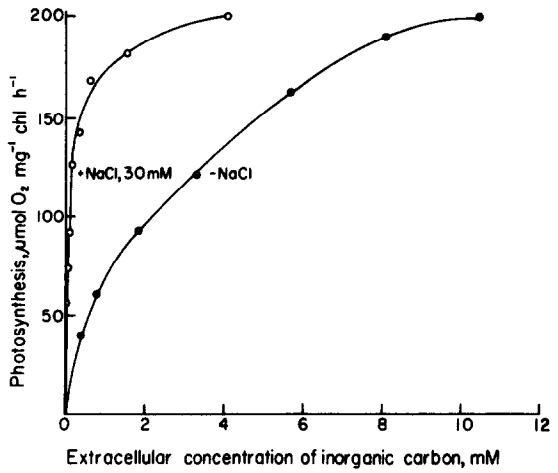


Fig.1. Rate of photosynthetic $O_2$ evolution as a function of external $C_i$ concentration in the presence and absence of $Na^+$. Light intensity was 7 mW·cm$^{-2}$ (400–700 nm), 30°C, ±NaCl (40 mM), pH 9.0.

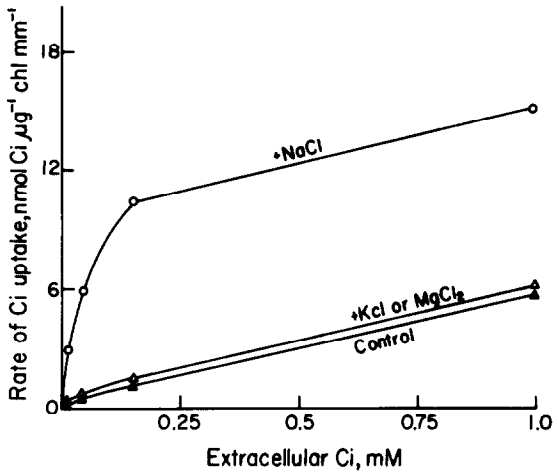


Fig.2. The effect of NaCl, KCl and $MgCl_2$ on the curve relating rate of $C_i$ uptake to external $C_i$ concentration. Cells were exposed for 5 s to the desired $^{14}C_i$ concentration in the presence or absence of the various salts (30 mM each). Other conditions as in fig.1.

medium depends on $HCO_3^-$ transport capacity [10]. Moreover, the oblique curve obtained in the absence of $Na^+$ resembles that earlier observed for cells adapted to high ambient $CO_2$ conditions. The lesser apparent photosynthetic affinity of such cells for $C_i$ in the medium, as compared with cells adapted to low ambient $CO_2$, is attributed to their lower capacity for $HCO_3^-$ transport [10]. These findings therefore suggested that the capacity for active $HCO_3^-$ uptake by *A. variabilis* may depend on the presence of $Na^+$ in the medium.

Data presented in fig.2,3 support this suggestion. Direct measurement of $C_i$ uptake from the medium (estimated over a time interval so brief that 90% of the $C_i$ absorbed was still in inorganic form) was strongly promoted in the presence of 30 mM $Na^+$. This effect appears to be highly specific for $Na^+$, as KCl, $MgCl_2$ and $CaCl_2$ (not shown) could not replace NaCl (fig.2). LiCl countered the NaCl stimulation, while $Na_2SO_4$ had an effect equivalent to that of NaCl (not shown).

Photosynthesis itself was not directly affected by the presence or absence of $Na^+$ at the concentrations used here, as can be seen when the photosynthetic rate (accumulation of acid-stable $^{14}C$) is plotted against the intracellular $C_i$ pool (fig.3). The points for control and $Na^+$-treated cells lie on the same line. It may thus be concluded that the lower apparent photosynthetic capacity for extracellular $C_i$ (fig.1) resulted from the reduced capacity for $C_i$ transport in the absence of $Na^+$ (fig.2).

Fig.4 gives the dependence of $HCO_3^-$ uptake on $Na^+$ concentration. The highest rate of $HCO_3^-$ uptake from a medium containing 0.15 mM $HCO_3^-$, was obtained in the presence of 40 mM $Na^+$.

One possible explanation for the alteration in the $K_m$ and $V_{max}$ of the $C_i$ transporting system by

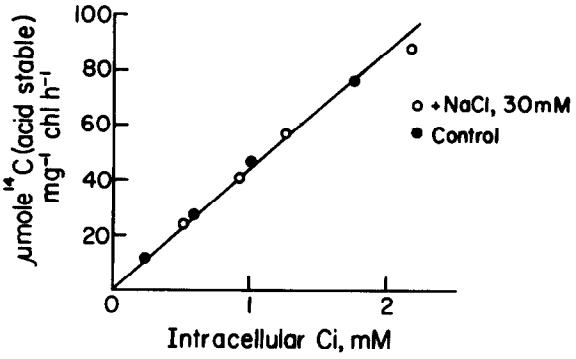


Fig.3. Rate of accumulation of photosynthetic products as a function of the intracellular $C_i$ concentration in the presence or absence of NaCl. Data calculated from the rate of accumulation of $^{14}C$ acid-stable products and the corresponding intracellular $C_i$ concentration in experiments such as the one presented in fig.2.

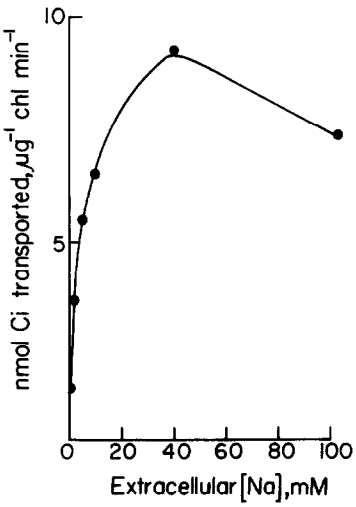


Fig.4. Dependence of $HCO_3^-$ uptake on $Na^+$ concentration. Cells supplied with 0.15 mM $NaH^{14}CO_3$ for 5 s. Other conditions as in fig.1.

$Na^+$ (fig.2, and in preparation) could be a specific effect of $Na^+$ on the $HCO_3^-$ porter, leading to altered $HCO_3^-$ binding parameters. However, bearing in mind the very large influx of $HCO_3^-$, which yields $OH^-$ within the cell, and the major role recently suggested for $Na^+$–$H^+$ exchange mechanisms in the maintenance of intracellular pH in bacteria [6–8] it seems likely that $Na^+$ might be required for the regulation of intracellular pH during $HCO_3^-$ uptake. This model would predict that addition of $HCO_3^-$ in the absence of $Na^+$ would lead to alkalization of the cytoplasm. There are various ways in which this local alkalization could alter the kinetic parameters for $HCO_3^-$ uptake, including a change in the rate of dissociation of the carrier-$HCO_3^-$ complex at the inner side of the membrane. This model would also predict that the unidirectional fluxes of $Na^+$ would show dependence on the presence of $HCO_3^-$; and the magnitude of these fluxes would be required to be as large as that of the $HCO_3^-$ flux.

Confirmation that the latter requirement is fulfilled could also support an alternative model. It might be postulated that the $HCO_3^-$ uptake process is in fact $Na^+$–$HCO_3^-$ symport. In this case the various criteria used to establish the connection between 'driven' and 'driver' substrates would have to be met [11,12].

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